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RELATIONS OF PARASITIC FUNGI TO THEIR HOST PLANTS

I. STUDIES OF PARASITIZED LEAF TISSUE

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(WITH NINE FIGURES)

Various phases of pathologic study have occupied the attention of botanists at different times. Before the exciting causes of diseases in plants were known, the general external appearance of the affected organ was described. Later, most of the attention was directed toward the discovery of the parasitic organisms which cause the derangements, and incidentally the study of the physiologic responses of the host was begun. Within the last few years many students of the subject have examined various morphologic changes which occur in diseased plants, first dealing almost entirely with the gross anatomic appearance, but later making more minute histologic and cytologic investigations. Leaf tissue, when invaded by fungi, however, has not been thus carefully studied. Moreover, comparative studies are always helpful in deciding general principles, and so it is in pathologic morphology. Only as we become acquainted with many examples of cytologic and histologic changes, shall we be able to approximate the truth regarding the reaction of the host plant to parasitic invasion. The practical value of such results can hardly be doubted. WOODS (**92**) has written of this matter as follows:

To most successfully combat a disease, we should know the causes that contribute to it, and as much about the causes as possible. We should under-

stand the pathological reactions of the diseased plant. Only in this way shall we be able to remove the causes or protect the plant against them or assist it to recover.

If, then, we desire to find a safe remedy, we must know all that is possible to know concerning the disease. As a link in the chain of evidence this paper is presented, with the hope that it may serve to extend knowledge of the reaction of leaf tissue to fungous invasion.

Historic

The relations between parasitic fungi and their host plants are of various kinds. The subject might be divided into two parts: (1) the changes in the fungi when grown upon various substrata, and (2) the effects of the fungus upon its host plants. Among the latter we can easily distinguish two classes, though one class is dependent upon the other; those changes which are disturbances of the physiologic processes, and those which are changes in the morphologic structure.

As we are to deal with the latter class in this paper, we shall turn our attention to the investigations on pathologic morphology which have previously been reported. The two phases which we must consider are (1) the anatomic and histologic, dealing with abnormal organs and tissues, and (2) the cytologic, dealing with abnormal cell structures.

Various pathologic modifications of the floral organs have been noted. MOLLIARD (**48**) described various changes in flowers caused by *Peronospora*, *Cystopus*, and other fungi, as well as by insects. WAKKER (**84**), in his most useful paper, reported additionally a number of abnormalities in some or all the organs of various flowers. The reproductive organs and corolla of two species of *Teucrium* are attacked and changed in structure by the larvae of *Copium* (HOUARD **29, 32**). Galls on the flowering parts of *Euphorbia Cyparissias* also were described by HOUARD (**30, 31**). The changes which WARD (**86**) cited as occurring in the buds of *Lilium candidum* are less striking but none the less interesting. The effects of *Cystopus candidus* upon the various organs of its host as described by EBERHARDT (**19, 20**) are notable.

TREUB (79) described the effect of *Heterodera* upon the root structure, and other writers have also studied abnormal root structures.

Stems and branches are also subject to invasion by parasites of various kinds. A somewhat detailed description of the deformation caused by *Ustilago* in the stem of *Zea Mays* was given by Miss KNOWLES (35), while WAKKER (84) showed that many groups of fungi have the power of changing the appearance and structure of stems. The Exoasceae cause numerous hypertrophies and other abnormalities in the vegetative organs as shown by SMITH (70). HARTMAN (27) described the witch broom of the white fir. Several of our common flowering plants, such as *Hepatica* and certain species of *Euphorbia*, were described by MEEHAN (43) as having elongated petioles and stems when attacked by rusts. EBERHARDT (19, 20) gave some histologic data regarding various stem tissues in pathologic condition. Some abnormal anatomic conditions were described by MOLLIARD (50).

The effects of disease upon the forms of leaves are among the most noticeable of the pathologic phenomena. WORONIN (93*a*) found various modifications of the leaf tissue caused by *Exobasidium Vaccinii*. *Exoascus* also causes very striking abnormalities in the leaves of various species of *Prunus* that have been described by Miss KNOWLES (34), ATKINSON (4), and SMITH (70). WAKKER (84) described various leaf modifications due to fungi. Some histologic changes of the needles of the witch broom of the white fir were noted by HARTMAN (27). The observations of PEGLION (63) showed that other kinds of rusts cause changes in the structure of leaves and stems. The anatomic changes caused by species of *Gymnosporangium* were described by WÖRNLE (94*a*); and ANDERSON (1) described the same for *Aecidium elatinum* on *Abies balsamea*. Plants growing normally in one range of temperature, when placed in a colder climate are often noticeably affected. BONNIER (6, 7), WAGNER (83), and HOUARD (28) have given us valuable results in their studies on this subject. Intumescences caused by abnormal environment have been described by Miss DALE (13, 14, 15), VON SCHRENK (67*a*), and others. The effects of various chemical substances upon plants have been studied. Of the work on this

subject we may mention that of WILFARTH and WIMMER (89, 90), CROCKER and KNIGHT (12), NĚMEC (59), and ANDREWS (3). Finally, we may note that wind has been found to play an important part in the production of abnormalities. Such observations were made by HANSEN (26*a*) and BRUCK (9). The general subject of "pathological plant anatomy" has been most satisfactorily reviewed by KÜSTER (38), who has also published various articles upon various phases of the subject, but especially concerning the anatomic features of gall tissues (37). This review of papers upon the subject of the histologic and gross anatomic changes, though incomplete, will serve to show the present extent of the subject, and through these references the rest of the papers may be traced.

From the following review of work upon pathologic cytology it will be seen that fungi, insects, poisonous substances, changes of temperature, and other physical forces all tend to modify the plant cell.

WORONIN (93*a*) found that *Exobasidium Vaccinii* on *Vaccinium Vitis-Idaea* reduces the amount of chlorophyll, and that the red-colored erythrophyll takes its place in the palisade cells. Miss KNOWLES (34) found that when peach leaves are attacked by *Exoascus deformans* the epidermal cells become rounded and have thickened walls, the palisade cells become nearly isodiametric, and the protoplasm is reduced in amount. ATKINSON (4) found that certain of the parenchyma cells become "very much elongated and curved or sinuous in form." TUBEUF (80) mentioned secondary cell formation in the palisade of *Populus niger* in leaves attacked by *Exoascus aureus*. In the cells of *Lilium candidum* affected by a *Botrytis* disease, WARD (86) found that the mycelium causes a swelling, dissolving, and discoloring of the cellulose cell walls, but does not directly affect the protoplasmic parts. The same observer (87) described similar effects of a *Botrytis* disease upon the snowdrop. In WAKKER'S paper (84) there are occasional references to cytologic phenomena. In most of the hypertrophied parts no chlorophyll is formed. Calcium oxalate is in the form of masses of small crystals ("Drusen") in the flower and leaf cells of *Rhamnus Frangula*, but is wanting usually in the parts attacked by *Aecidium*. In other cases numerous small individual crystals

are formed. Starch is often abundant in certain hypertrophies. Bacteria were reported by DANGEARD (**16, 17**) as causing the swelling of the nucleus of *Euglena*, the disappearance of the nucleolus, and the disorganization of the chloroplasts. The effect on the contents of cells in the process of fermentation has been studied by MATRUCHOT and MOLLIARD (**46**), and PEIRCE (**64***a*) has described the changes in the root tubercles on the bur clover. *Cystopus candidus* usually causes an unusual deposition of starch and formation of chlorophyll in parts usually free from these substances, as described by EBERHARDT (**19, 20**). Other students, as GRANT SMITH (**69**), NORDHAUSEN (**61**), W. G. SMITH (**70**), WÖRNLE (**94***a*), HALSTED (**25***b*), MIYOSHI (**44***b*), and HARTIG (**26***b*), have noted the effects of fungous invasion upon the cellulose walls, and the distribution of starch and of calcium oxalate. The cellulose walls may be thickened or dissolved, and the starch and calcium oxalate content may be increased or decreased as determined by the season, the specific invader, and the host. WARD (**88**) has found that in the wheat plant the hyphae of the invading rust do not seem to affect the "chlorophyll-corpuscles or the nuclei until a late stage of growth." The leaves of the witch broom of the white fir are described by HARTMAN (**27**) as having small amounts of chlorophyll and starch. WÖRNLE (**94***a*) reports that *Gymnosporangium* causes excessive nuclear division and cell formation in pine needles. The Ustilagineae, according to STROHMEYER (**75**), cause various changes in the cells of the host, both hypertrophy and hyperplasy of the parenchyma occurring. MOLLIARD (**48**) described a number of cytologic changes caused by insects and fungi growing on various hosts. *Cystopus candidus* causes the cells to assume abnormal forms and sizes, the nuclei to enlarge, and the normal chlorophyll content to change. *Peronospora* does not affect its hosts in this way. *Puccinia Violae* on *Viola silvestris* causes the nuclei and the nucleoli to enlarge, and the power of division of the former to increase. The petals of *Euphorbia Cyparissias* when attacked by *Uromyces scutellatus* and *U. praeminens* show cells with enlarged nuclei and chlorophyll formation.

The effects of various insect parasites upon the vegetative cells may be summed up in general thus: nuclei and nucleoli

enlarged, chloroplasts reduced in size, often a more abundant protoplasm than normally, and variations in the calcium oxalate content. In some fungous galls GUTTENBERG (**24**) found a tendency for the nucleus to become lobed, to divide amitotically, to decrease in size, and to force the chromatin toward the periphery. The cytologic features of some gall tissues, resulting from insect invasion, has also been described by MOLLIARD (**49**). The nuclei show a decided tendency toward amitotic division, which often results in the presence of several nuclei in a cell, with no formation of new walls. The nucleoli as well as the nuclei become greatly hypertrophied, and the former may be divided often without the subsequent division of the nucleus. At times the nucleus multiplies by a method of budding in addition to the more common means of abstriction. The nuclear membrane may finally disappear, and eventually even the nucleoli may become disintegrated. HOUARD (**29**) reported similar hypertrophy of the cell organs of the flowers of *Teucrium* when attacked by the larvae of *Copium*. PERCIVAL (**64***b*) described the effect of *Synchytrium* in the potato "wart disease" upon the host cells. These enlarge, the cytoplasm increases, and the nucleus becomes deformed. "The organisms stimulate the invaded cells and at the same time appear to stimulate division and growth in the adjoining cells." Changes in root cells have been noted from time to time, especially in the studies on mycorhizae. MAGNUS (**41**) found the nucleus modified in these symbiotic structures. SHIBATA (**68**) notes that the nuclei in such conditions become enlarged, amoeboid, and divide amitotically. They may be strongly colored at first, but later they seem to become normal as regards division and color. ZACH (**94***b*) describes the following cytological changes in cycad root cells due to fungous invasion. The nucleus becomes amoeboid or otherwise misshapen, the starch is dissolved away, the calcium oxalate increases in amount, and finally nucleus and plasm die. The cells of the growing point and the *Anabaena* region are not seriously invaded. MOLLIARD (**52**) reported that the nematode worm lives in a tissue which has giant cells with numerous nuclei and enlarged nucleoli. VUILLEMIN and LEGRAIN (**82**) reported various nuclear phenomena caused by the same worm in symbiotic relationship with the roots

of plants cultivated in the dry Sahara region. MOLLIARD (**51**) found "nuclear protoplasmic division" in stems attacked by *Phytoptus*. NAWASCHIN (**54**) described strongly hypertrophied cells, and enlarged nuclei with poor chromatin content, in tissues invaded by *Plasmodiophora Brassicae*. TOUMEY (**77**) noted that the nucleus of a crown-gall cell becomes much enlarged and finally "appears as if eroded on the surface." The nucleoli are very persistent. Under abnormal conditions the root tip of *Allium Cepa* shows enlarged cells and nuclei (some lacking nucleoli), and often an increased amount of chromatin. NĚMEC (**56**) also noticed nuclear fragmentation in the same root tips. The nuclei in leguminous root tubercles are reported by PARATONE (**62**) as becoming amoeboid and abnormally colored; CHODAT (**11**), however, noted no great changes.

The effects of changes of temperature upon cell structure have also been studied to some extent. PRILLIEUX (**65**) grew seedlings in heated soil, and found that the nuclei became numerous and variable in form. They increased by fragmentation and often possess numerous nucleoli of various shapes and sizes. These are vacuolated. NĚMEC (**57**) found that nuclei assume amoeboid forms in lowered temperatures. SCHRAMMEN (**66**) found that abnormal nuclear division ("pseudomitosis"), abnormal size of nucleus, and abnormal mass of nucleolin and kinoplasm resulted from changing the temperature surrounding the growing point of a *Vicia Faba* stem. MATRUCHOT and MOLLIARD (**45, 47**) give very detailed descriptions of abnormal nuclei produced at freezing temperatures, especially noting the distribution of chromatin. NĚMEC (**58***a*) produced multinucleated cells in the roots of *Vicia Faba* by placing them in a 1 per cent copper sulphate solution; upon returning them to normal conditions karyogamy took place and uninucleated cells were formed. GRANT (**23**) has reported upon various multinucleated cells. ANDREWS (**2**) found that a nucleus, deprived of its nucleolus can survive for a long time, but a new nucleolus is not formed. The influence of benzene gas upon cell formation was studied by BLAZEK (**5***a*), who reported that simultaneous nuclear division takes place, and many daughter nuclei are formed either with or without subsequent cell wall

formation. WASIELEWSKI (85) and WISSELINGH (91) have discussed the question of amitosis in various tissues. Miss DALE (13, 15) found that in certain intumescences caused by abnormal light, heat, etc., oil is formed in place of starch, the nuclei become club-shaped and highly refractive, and the nucleoli are often increased in number. Amitosis was found to be almost universal, and formed nuclei of unequal size. VON SCHRENK (67*a*) described similar intumescences due to chemic stimulation. Other studies upon the structure of intumescences were made by SORAUER (71, 72) and STEINER (73). The structure and the pathologic modifications of chromatophores have been studied by KÜSTER (39) in *Ceramium* cells, where under various influences they may be contracted into drops or flattened out into irregular bands. NĚMEC (55) has reported the decrease in the number of chromosomes in old tissue, and their increase in hypertrophied cells. Besides these already enumerated, it would be well to note the following as articles dealing with several phases of the subject, and containing valuable lists of references: ZIMMERMAN (96, 97), WARD (87), UNGER (81), FAIRCHILD (21), and NĚMEC (60). Some abnormal nuclear phenomena have been described also by MIEHE (44*a*) near wound tissue, KOHL (36) in cells under the influence of asparagin, and ZACHARIAS (95) under various influences.

Summing up the results so far obtained in the study of the effects of parasitic invasion and abnormal physiologic influence upon the histologic and cytologic elements of plants, we find that the various kinds of tissues, collenchyma, parenchyma, sclerenchyma, and cork, may be abnormally developed or repressed; cell walls may be simply perforated, or much thickened and more or less changed in constitution; secondary cell formation may arise; nuclei and nucleoli may be increased or reduced in number and size, and variously deformed; chromatin may likewise be increased or decreased; the cell sap may acquire a new color; the starch and calcium oxalate content may change; and the chromatophores changed in appearance and efficiency.

Methods

For the investigations to be reported here the ordinary methods of preserving the material were used. The medium solution of

chromacetic acid was found to be the most useful killing and fixing fluid experimented with. Picric alcohol was less satisfactory, because stains of several kinds refuse to affect the tissue when preserved in this solution. For the same reason, picronigrosin was not very successful. An abundance of material was usually kept in the dry condition as herbarium specimens, and some was preserved as well in 4 per cent formalin. Paraffin, melting at about 52° C., was used for most of the work, though the harder grade, melting at 60°, was employed for some of the tough, resistant leaf tissues.

The orseillin-anilin blue method of staining, as outlined by STRASBURGER (**74***a*), was used for all of the preliminary work. This combination serves to differentiate the fungus in the host tissue, and also to make it easy to distinguish the cell contents. The latter stains rather darkly with orseillin, and the host cell walls lightly with the anilin blue. The fungous walls stain much more deeply with the anilin blue. Later in the work, when studying the nucleus, Haidenhain's hematoxylin was used, about as outlined by CHAMBERLAIN (**10**). The differentiation thus obtained was very satisfactory, since the nucleus held the stain more tenaciously than did the cell walls. Fuchsin also was used to some extent.

About 50 different specimens were preserved, sectioned, and examined. Many of these failed to be of value, either because of the great destruction of the host tissue by the fungus, or because of the absence of distinctive and recognizable cytologic changes. The drawings were all made with a camera lucida; and a $\frac{1}{12}$-inch, achromatic oil immersion objective was used for all high magnifications in all camera drawings. The drawings, with one or two exceptions, are of the same magnification, and hence can be compared directly with one another. The attempt was made to get satisfactory material which would illustrate as great a diversity of host plants as well as of fungi as possible. In the following pages, therefore, there will be representatives of both monocotyledons and dicotyledons, and of the latter from several diverse families. The Uredineae, the Ustilagineae, the Phycomycetes, and the Fungi Imperfecti are all represented. The descriptions

of normal and diseased material are from the slides prepared by the methods above mentioned. The normal tissue was cut from the same leaf as the diseased, unless the leaf showed in all parts the effect of the parasitism. In this case a leaf close to the diseased one was chosen in order to have comparable material.

Observations

1. Gaylussacia baccata (Wang.) C. Koch.—The cause of the disease is not clear. The diseased area is blackest on the upper side, and a white deposit is found on the lower surface of the leaf. The normal leaf tissue is as follows: (1) a single layer of nearly isodiametric cells in the upper epidermis; (2) a single layer of palisade cells; (3) a rather loose, spongy parenchyma with comparatively large intercellular spaces; and (4) a lower epidermis of cells usually somewhat smaller than those of the upper epidermis. The plant studied was collected in early summer, and hence the leaves were fully formed, but still in a vigorous condition. The location of the plant was at the edge of a wooded area, but open, so that the leaves received an abundance of light. The leaf cells have a thin peripheral lining of protoplasm, in which the chloroplasts and the nuclei are imbedded. The cuticle is nearly colorless, and much thicker over the upper than over the lower epidermal cells.

The parasitized leaf shows a number of changes. Just before the palisade cells and after the sponge cells begin to collapse, they are filled with a uniformly brown-stained material. Previous to this they are filled with a granular mass which is stained yellow-brown. In both of these stages of degeneration there are neither chloroplasts nor nuclei to be seen. At the same time, the upper epidermal cells become considerably elongated, and the cuticle is light yellow to brown. In the last phase of degeneration the epidermal cells become completely collapsed, the palisade cells are much shrunken, and the sponge cells have practically disappeared, while the cell walls are uniformly brown, and the cells empty.

2. Viola cucullata Ait. (?), parasitized by *Puccinia Violae* (Schum.) DC.—The normal leaf tissue of this plant is rather poorly differentiated. The epidermal cells are very irregular in shape and size, and the cuticle is thin. The mesophyll is composed of a loose

mesh of rounded cells, stretching from one epidermis to the other. The tissue is evidently very easily ruptured, and is seldom complete except at the veins and in connection with the fungous spore beds. The plants examined were growing in a well-shaded and damp location, and were collected in early summer.

In the parasitized regions the cells are closely compacted, and more or less bound together by the invading mycelium which fills the intercellular spaces. Here also the hematoxylin stains much more intensely than in the normal tissue. There seems to be some hyperplasy of the mesophyll. The epidermis is ruptured by the

FIGS. 1–3.—Cells from leaf of *Viola cucullata* parasitized by *Puccinia Violae*: fig. 1, two cells showing deformed nuclei and increased number of nucleoli; fig. 2, cell showing three nuclei and a few chloroplasts; fig. 3, cell showing three deformed nuclei.

spore bed, but the influence of the fungus does not extend far beyond this radius. The nuclei, which are not prominent in the normal cells, become somewhat enlarged, and increase in number in the cells. There are numerous instances where the number is increased to two, while some cells were noticed that contained three nuclei. The nuclei are also more or less deformed, varying from nearly circular in cross-section to oval, oblong, or slightly pear-shaped. The chloroplasts seem to be little affected, at least in shape and size, by the presence of the parasite. Fig. 1 shows some examples of the deformations to the nuclei which are found in the cells near the

spore bed of the rust; fig. 2 shows the abnormal number of three nuclei in one cell; while fig. 3 shows some deformation accompanying the hyperplastic condition.

3. PSEDERA TRICUSPIDATA (Sieb. and Zucc.) Rehder, parasitized by *Phyllosticta Labruscae* Thum.—The normal leaf tissue is similar to that of the *Gaylussacia* described above, and the pathologic changes are very much alike in the two plants. The browning and collapse of the epidermal, palisade, and sponge cells are common to both. The early disappearance of nuclei and chloroplasts is also similar in the two. There is in this case a granular protoplasm in the cells next to the dead area, while the homogeneous brown mass is not formed. The sponge tissue is more completely disorganized than the palisade. This plant, collected in early July, was growing upon a wall exposed to the morning sun.

4. SMILAX GLAUCA Walt., parasitized by a member of the Phaeodidymae of the Sphaerioidaceae.—The leaf parenchyma is not differentiated into palisade and sponge tissues. The diseased area is badly shrunken and broken down. The fungous perithecia are scattered irregularly over this dead area. Because of the thorough killing of the host tissue, no special cytologic changes could be noted. At the edges of the diseased area the cells of both the upper and lower epidermis are turned brown to black, while some of the mesophyll cells are filled with a uniformly brown-staining material. The intercellular spaces and the primary lamellae are often stained very deeply by the hematoxylin. In this transition region no cell organs are visible, and whatever is left of the protoplast is turned black or brown. This coloration sometimes extends to the cell walls as well.

5. POTENTILLA CANADENSIS L., parasitized by *Puccinia Potentillae* Schw. (?).—There is a double parasitism represented here. *Darluca filum* was found growing abundantly upon the spore beds of the rust, and at times apparently directly upon the leaf tissue of *Potentilla*. This latter condition, however, may have been due to the rust mycelium in the tissues which had not yet produced a noticeable spore bed.

The normal structure of this *Potentilla* leaf is somewhat more complex than that of the *Viola* described previously. The palisade

cells have a more oblong longitudinal section than the sponge cells and are more closely packed. The plants collected were growing in a place which was shaded all of the afternoon and part of the morning. The collection was made about the middle of July.

When the leaf is attacked by the rust, the epidermis is broken, the palisade is poorly developed, and more or less replaced by rounded cells common to the sponge and the diseased palisade tissues. In the early stages of the disease the epidermis separates from the mesophyll. There is some hyperplasy of the sponge tissue. The cells, which remain in the tissue directly under the spore beds, are widely scattered and separated by the mycelium of the rust. When the rust is parasitized by *Darluca* the palisade tissue is less abnormal and disorganized. The chlorophyll seems to have largely disappeared from the cells which are within the influence of the rust. A similar effect upon the nuclei is usually seen. An increase in the number of nuclei in individual cells was noticed, however, in tissue just below a perithecium of *Darluca*, which was found imbedded directly in the tissue of the *Potentilla* leaf. Fig. 4 shows two contiguous cells, each having two nuclei; there is also a distinct deformation of these double nuclei.

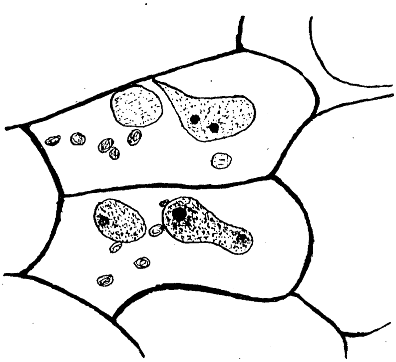
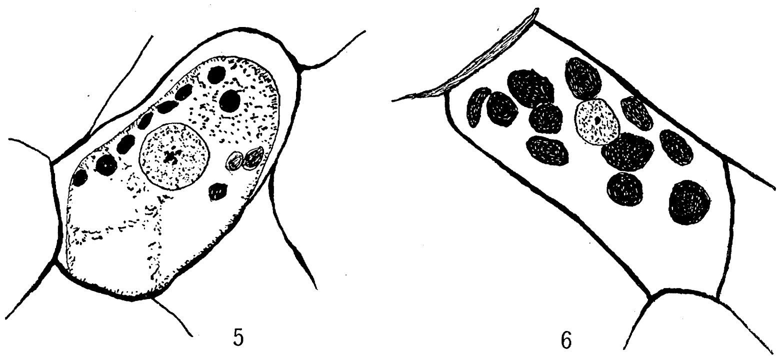


FIG. 4.—Two cells from *Potentilla canadensis* leaf parasitized by *Puccinia Potentillae*, showing two nuclei in each, and in one a suggestion of the beginning of an abnormal division of one of the nuclei.

6. PANICUM LATIFOLIUM L.—The cause of the disease upon this *Panicum* is not clear, though it is surely of fungous nature. The normal leaf tissue is that of the ordinary grasses. The epidermal cells on both sides of the leaf are very large as compared with the simple mesophyll cells which occupy the space between the two layers of epidermis. In the healthy chlorophyllose cells there is only a thin peripheral lining of protoplasm in which the nuclei and the chloroplasts are distributed.

Many of the cells of the diseased area are filled with a homogeneous substance which stains blue, so that at a glance the location of the diseased tissue can easily be discovered. The protoplasm, which is somewhat more abundant and more evenly distributed in the diseased cells than in the normal, is very finely granulated. Many of the diseased cells show two nuclei. All of the nuclei in the vicinity stain deeper or retain the stain more tenaciously than in the healthy tissue, and are relatively larger than in the normal cells. The chlorophyll has largely disappeared from the cells close to the point of invasion, but farther away the



FIGS. 5, 6.—Cells from leaf of *Panicum latifolium:* fig. 5, cell from a diseased leaf, showing an enlarged nucleus and diminutive chloroplasts; the cell is slightly plasmolyzed; fig. 6, cell from an unaffected region, showing a normal nucleus and normally sized chloroplasts.

chloroplasts are merely reduced in size. Figs. 5 and 6 show enlarged and normal nuclei respectively; the difference in the size of the chloroplasts is easily seen also.

7. PYRUS MALUS L., parasitized by *Gymnosporangium* sp.—The structure of this leaf is somewhat more complex than any of those previously described. The upper and lower epidermal cells are in single layers, and are partly isodiametric and partly oblong in shape. The palisade cells, which are in two layers, have their nuclei symmetrically placed in the peripheral protoplasm close to the middle of the lateral walls. The chloroplasts are also arranged along the same walls. The cells of the upper palisade layer are about a third longer than those of the second layer, and are about the

same width as those cells. This palisade occupies half the thickness of the leaf. The sponge cells are loosely connected and have large air chambers scattered among them. The tree, from which the diseased leaves were taken, was growing in a thicket, and about 50 feet from a *Juniperus* tree.

The portion of the leaf which is parasitized is about twice as thick as the normal tissue. This thickening is due to two factors, the hypertrophy of the sponge tissue, and the presence of masses of mycelium. The palisade cells are least affected. The cells in the upper layer are shortened until they are about the length of the cells in the second layer. The space thus left between the upper epidermis and the palisade cells is occupied by mycelium from which the pycnia are developed. The upper epidermis is puffed up and ruptured, the cells are nearly collapsed, the walls are changed to a brown color, and the cuticle is mostly destroyed. The parenchyma cell walls seem to remain about normal. The sponge cells are enlarged to twice or thrice their normal diameters, and the spaces between are filled with the heavily stained mycelium. The nuclei of the sponge cells are the first to show the effects of the presence of the fungus by becoming rather larger than they are normally. They are not, however, otherwise materially changed. Many cells are partially filled with a yellow, granular deposit (fig. 7).[1]

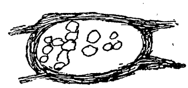


FIG. 7.—A diseased cell from a leaf of *Pyrus Malus* parasitized by *Gymnosporangium*, showing no contents except irregular yellowish granules.

8. SMILACINA RACEMOSA (L.) Desf., parasitized by *Phyllosticta cruenta* (Fr.) Kicks.—The leaf of *Smilacina* is rather simple in structure. The upper epidermis has relatively large oblong cells, interspersed at intervals with short cubical cells. The lower epidermis has smaller and usually more regular cells. Between these two layers is a rather loose parenchyma with large air spaces, and with the larger number of cells close to the upper epidermis. The plants collected were growing at the side of a road through the woods, where the light was rather weak and there was abundant moisture.

[1] Fig. 7 was drawn with a $\frac{1}{6}$-inch objective, and hence is not on the same scale as the other figures.

In the diseased area the cuticle and the epidermal cells are affected to a greater distance from the center of infection than the sponge cells. They are early turned brown and the epidermis is shrunken. The sponge tissue is badly disorganized, but chloroplasts and nuclei are present. The latter attain a larger size, and have a light brown color, whereas when normal they are easily stained with hematoxylin. They often have two nuclei and a granular plasm. Later, in the degeneration of the nucleus, the nucleoli disappear and the nucleus stains deeper. The chloroplasts may later disappear and afterward the other constituents of the cell. Fig. 8 shows the light brown enlarged nuclei in cells affected by the parasite.

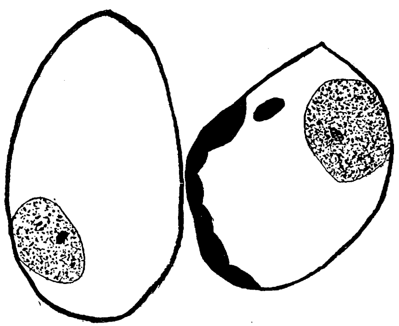


FIG. 8.—Two cells from a leaf of *Smilacina racemosa* parasitized by *Phyllosticta cruenta*, showing enlarged nuclei.

9. CASTANEA DENTATA (Marsh.) Borkh., parasitized by *Cryptosporium epiphyllum* C. and E.—The normal leaf is covered by an epidermis with cells rather larger than those of the sponge tissue. The palisade tissue, which is composed of very long narrow cells, sometimes with a row of short cells below, occupies about half of the total thickness of the leaf. The sponge is very loose and has large air chambers. The cells of the lower epidermis are smaller than those of the upper.

As examined in prepared material, the diseased area is very sharply separated from the healthy part of the leaf. There are practically no cells that show transition phases between the normal and the diseased conditions. The epidermal cells over the parasitized portion are flattened, and often filled with granular deposits which have also been noticed in other diseased plants. The palisade and sponge cells are much shrunken and the entire contents were killed apparently before the specimen was collected. The entire diseased area is yellow-brown, while the normal tissue stains easily with anilin blue. No cytologic changes are to be seen except the deposit of the yellow-brown material throughout

many of the cells, and the occasional enlargement of the nucleus. In some cases the palisade tissue has thus been affected, while the sponge cells are less noticeably changed.

10. XANTHIUM CANADENSE Mill. (?), parasitized by *Puccinia Xanthii* Schu.—The normal leaf tissue is very loosely formed between the upper and the lower epidermal layers. The palisade is scattered, and the sponge is permeated by very large air chambers. Transitional phases between the normal and the diseased conditions of the host cells were not found.

This tissue is more profoundly altered than any other under discussion. It is in many places almost completely replaced by the fungous mycelium. The cells which remain have no protoplasm and are filled with oil globules. On both the upper and the lower leaf surfaces the mycelium is abundant and the telial spores are very numerous. Within the mixture of parenchyma cells and mycelium, which replaces the normal tissue, there are cystlike bodies which are composed of masses of mycelium. These objects are hollow spheres, and from the inner surface arise telial spores exactly similar to those borne in the normal way upon the exterior of the leaf. Part of the mycelium near the exterior stained brown, while that within the host tissue stained blue with anilin blue.

11. ZEA MAYS L., parasitized by *Ustilago Maydis* (DC.) Tul.—The normal host cells are not changed in general appearance, and the general structure of the leaf remains practically normal. Very early in the disease the chloroplasts disappear, but the nuclei remain until very late in the formation of the spores by the smut. In some cases the number of nuclei in a cell is increased, two being the largest number noticed. In this condition the nuclei may also be slightly reduced in size. The nuclei often become deformed, varying from globular to pear-shaped or even crescent-shaped. This deformation seems to be due to mechanical forces at least in part, for in the first place there are cases in which the nucleus is pressed upon on all sides by the forming spores, and the nucleus conforms itself to the rounded shape of the spore walls; in the second place there are nuclei in the diseased area and even in the cells being filled with spores which are normal in shape. If the deformation were due to chemic stimulation, this latter condition

would hardly obtain. The epidermal cells over a diseased region are often filled with a yellowish granular deposit. In the diseased area the cells of the vascular bundle and of the parenchyma alike become filled with a uniform gray plasm, which later breaks up into rounded or hexagonal areas as shown in fig. 9. In the latter stage of the disease these attain more clearly defined walls. It seems evident that these develop eventually into the fungous spores, though the material at hand was not quite old enough to show the last stage of the transformation into the spores. No mycelium was found in the diseased tissue after the formation of the areas mentioned above had begun. It would seem from this that after a certain stage in the disease the fungous mycelium passes into a plastic stage within the host cells, and that then the plastic mass breaks up into spores as previously described. Miss KNOWLES (**35**) states, in her study of the effect of this same fungus upon the corn stem, that the vegetative mycelium breaks up into masses of smaller filaments which swell up, "except that in many cases, indeed in most cases, the filaments lose their individual form and are more or less blended in a gelatinous, shapeless mass." This gelatinous mass fills the host cells at least in the leaf tissue.

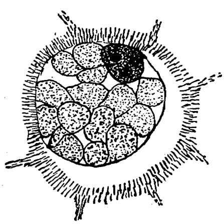


FIG. 9.—One of the outer elements of a vein in a leaf of *Zea Mays* parasitized by *Ustilago Maydis*, showing nucleus and early stage in spore formation within the cell.

12. RAPHANUS SATIVUS L., parasitized by *Albugo canadensis* (Pers.) Kuntze.—The fungus was growing on the cotyledons of young seedlings which were grown in the greenhouse. There is no regular structure to the parenchymatous tissue, as it is all of the ordinary sponge type. There is apparently no great hypertrophy or hyperplasy of the tissues. The blisters seem to be caused by the formation of the mycelium, conidiophores, and conidia just below the epidermis. Few, if any, changes in the cell organs are to be seen. The mycelium is found in abundance. The effect of this fungus seems to be merely that of starvation of the host, not that of poisoning it.

Discussion and conclusions

The changes which are caused in leaf tissue by parasitic fungi are similar to those which have previously been reported as occurring in other parts of phanerogamic plants, and caused by insect invasion, changes of temperature, and parasitic fungi. A comparison of the observations reported in the latter part of this paper with those of other workers reviewed in the earlier part will show that the enlarging, changing of form, and dividing of the nucleus, the changing of the composition of the cell walls, the reduction in the amount of chlorophyll, and other changes in the normal content of the leaf cells are all duplicated in other organs affected by destructive agents.

These changes are usually only variations of natural processes, and are not phenomena that are known only in pathologic tissues, for it is to be noted that these changes in the appearance of the nuclei are not unknown in normal vegetable cells. JOHOW (**33**) describes amitosis as occurring in the older cells of *Chara foetida*. The presence of two or more nuclei in a cell has been noted by TREUB (**78**) in bast cells of various plants; while a process of division is reported by VON BRETFELD (**8**) and MASSART (**42***a*) as so common in wound tissues that they believed nearly all such tissue is formed by the "amitotic" process, as they called it. More detailed work is necessary to show whether the amitotic process of JOHOW and the processes of nuclear division in wound and disease tissues are really analogous, since the former process seems to be for the purpose of increasing the nuclei in connection with the metabolism of mature cells, and the latter processes often lead to an increase in the number of cells, and are found in cells apparently stimulated to a kind of rejuvenescence. SCHÜRHOFF (**67***b*), moreover, has shown that this idea is far from correct, since there are many cases of mitosis in wound tissue, and no true amitosis is certainly known in these tissues. A process that appears to be true amitosis has been reported by SHIBATA (**68**) in mycorhizal cells, and by TISCHLER (**76**) in *Heterodera* galls on *Circaea*. A rather detailed discussion of this question is given by Miss DALE (**15**); while KÜSTER (**40**) and STRASBURGER (**74***b*) review the whole subject of the direct and indirect methods of nuclear division.

No nuclei were noted in actual process of division, but in a few instances there is some evidence of abnormal division. In *Potentilla* (fig. 4) such is the case. The nucleus is somewhat elongated, and at one end is stretched out into a conical point. A comparison of fig. 4 with HÄCKER'S (**25***a*) figs. 9, 10, 14, and 16 will show the close resemblance between the nuclei supposed to have divided recently (fig. 4) and the actual process as reported by HÄCKER. A case which is less clearly related is seen in fig. 1, where the nucleus is pear-shaped. Whether or not these are cases of true amitosis or of pseudoamitosis could not be determined, as only the end products were seen.

The composition of the cell walls of the host may be changed. Two evidences of this are forthcoming. In *Panicum*, especially, it was noted that the walls of the parasitized cells are more deeply stained than those of the ordinary cells. Since the walls are not noticeably thickened, the only remaining explanation is that some change in the chemical or physical composition of the walls has taken place, in such a way that they have a greater affinity for the stain, anilin blue. In most of the diseased tissues the walls become brown. This color is probably due to the formation of tannins. There is always more or less of these substances in the walls, and when the cells begin to die, it is known that this browning often takes place through the work of the tannins. In several cases, also, granular deposits were found in various cells in the diseased regions only (of *Castanea*, *Pyrus*, etc.), similar to those described by ZIMMERMAN (**98,** § 207). Just what would cause this precipitation is not clear.

It was not always possible to determine which constituents of the host cell first showed the effect of fungous invasion. It is evident, however, that there is no general rule to be laid down. In *Smilacina*, for example, the chloroplasts seem to disappear before any of the other cell contents, while in *Pyrus* the nuclei of the sponge cells become enlarged before there are any other signs of change. It has already been pointed out that in some cases the cuticle and epidermis are affected first, as the fungus spreads from the center of infection, while in other cases no such result was observed.

The effects of the rust upon the leaf tissue are similar to those of gall-producing insects. The nuclei are enlarged, the protoplasm is often increased, and there is considerable hypertrophy. In other words, the rust seems to stimulate the tissue rather than to retard its growth. The witch brooms are evidences of such an influence, and MOLLIARD (49) describes such effects caused by insects in several hosts.

The effects of the fungous invasion upon the protoplast are various. At times the nuclei may entirely or almost entirely disappear from the diseased areas, leaving the cells, thus deprived of the nucleus, in a dead condition. This was noted in *Potentilla* and *Gaylussacia*. In other cases there is an unusual activity in the nuclear divisions, resulting in several nuclei in one cell. *Viola* and *Panicum* have already been cited as examples of this. The fact has already been given that the nuclei may also become larger than normal. The chromatin content of the diseased nuclei seems at times to become greater also. Such a condition is seen in *Panicum*, in which the nuclei of the diseased cells stain more intensely with hematoxylin than those of the normal cells. This may not be due to an actual increase in the chromatin, but either to a relative increase in the nuclear acid, or to a physical rearrangement of the chromatin. The chloroplasts may be reduced in size and finally disappear, as in *Potentilla*, or they may persist, as in *Viola*, until the final disintegration of the cell. The differences in the reactions of the cytoplasm and of the cell walls have already been cited in other connections.

With the attacks of parasitic fungi on leaves the effects are varied, depending upon the species of the host and of fungus. The virulence of the parasite and the degree of resistance of the host are the chief factors involved. If the leaf, while in actively growing condition, is attacked by the fungus, the changes in the host are often profound, if the fungus is able to maintain itself against the protective measures put in operation by the host. The cytologic changes already described are likely to occur. The leaf curl of peach caused by *Exoascus deformans* is an example of this type of change. Miss KNOWLES (34) has examined the leaf structure in this disease and has found that the tissue is greatly altered

by the presence of the fungus. The leaves are attacked in the bud and very soon after their emergence (DUGGAR 18). Cytologic changes may also occur when the fungus acts slowly for any reason. If the fungus is a weak parasite or if the host is not the best suited to the fungus, the host will have time to react to the fungus, and notable changes may be expected, both in the cell and in the tissue as a whole. That there is a difference in the speed with which various parasites work is seen from the fact that some diseased tissues show cells in various degrees of degeneration or death, while in others there is no intergrading condition between the dead, brown cells of the diseased area and the normal cells of the healthy tissue. The *Gaylussacia* described above shows relatively slow action of the parasite, as is proven by the two following conditions. First, the cells react by showing a granular protoplasm, then a uniform mass of broken-down protoplasm, which is stained brown, and finally nearly empty cell walls. It is improbable that this gradual degeneration is merely a process that would normally take place in cells which are breaking down, because often, as in *Castanea*, none of these intergrading forms are found. Second, it is to be noted that in *Gaylussacia*, and other hosts reacting similarly, the cuticle and the epidermal cells are often affected before the mesophyll, as the fungus spreads from the center of infection. This tends also to prove that the fungus acts progressively and slowly enough to be observed in the successive steps. *Psedera*, *Viola*, and *Panicum* act somewhat similarly in that they show changes in the size and number of nuclei, etc., as cytologic changes induced by the slow action of the respective parasitic fungi. It should be noted in passing, also, that in the case of the rust which was parasitized with *Darluca*, there was a different effect produced upon the nuclei of the leaf cells from that produced by the unparasitized rust. In the latter case the nuclei seem to have disappeared, while in the former case the nuclei are present but very abnormal (fig. 4). It is perhaps significant that nuclei were found only in places where the rust was parasitized, and presumably lowered in its vitality. If the latter statement is true, then the rust would not be able to act so quickly as normally, thus giving the host time to react to its influence.

In some of the diseases described, in which the tissue is killed and badly disorganized, few if any cytologic changes are to be noticed. The reason for this seems to be that the virulence of the fungus is relatively so great that the cells of the host are killed without having time to react to any stimulus. In such cases, as in the *Castanea*, all tissues seem equally affected, and there are no transition cells from the healthy to the dead portion. Another factor also which may operate is that the host tissue is old, and has not the power of reacting rapidly. This is in contrast with the young, highly resistant tissues, which, if parasitized, are able to react rapidly, and so present abnormal nuclear and other cytologic phenomena. *Smilax* also is an example of a diseased tissue which shows no cytologic changes.

A question constantly before the student of pathology is whether there is any way of judging the degree of susceptibility of a plant to disease. First of all it must be remembered that a plant may be easily attacked and injured by one disease-producing organism, and be quite immune to the attacks of another. Leaving this phase of the question, however, we ask, "Is the amount of hypertrophy a true indication of the degree of parasitism?" If we imply in the word "parasitism" some influence derogatory to the welfare of the plant, we are almost forced to answer the question in the negative, for in cases of symbiosis there is often great hypertrophy.

It is difficult at present to draw a sharp line between parasitism and symbiosis, since similar effects upon the host are noted in both. Thus, in some of the most noted instances of symbiosis, the mycorhizas, there is often considerable hypertrophy. Moreover, many kinds of galls do not seem to injure the host beyond the local tissue, and here again there is great hypertrophy. Hence, taken alone, an excessive enlarging of a portion of a plant is not sufficient evidence of the degree of parasitism. But, taken in conjunction with the cytologic changes induced in the host, hypertrophy becomes a valuable diagnostic feature. It must not be forgotten, however, that even in such cases the parasitic nature of the organism is not proved, for cytologic changes in mycorhizas have been reported, as noted heretofore. Evidence has previously been given in regard to the diagnostic value of the cytologic changes

in such tissues as the leaves of *Pyrus* and *Xanthium*. Here the hypertrophy is coincident with the changes in the constitution of the cell, and it indicates a rather strong parasitism. On the other hand, as in *Psedera* and *Gaylussacia*, the virulence is so great that the cytologic changes are simply the fleeting features of a rapid degeneration, and of course no hypertrophy could take place. Here again the condition indicates a strong parasitism. TUBEUF (**80**) points out that the degree of susceptibility of the host, or in other words the degree or strength of parasitism, is indicated by the amount and kind of deformation, as has just been indicated. He draws his illustration from the Uredineae. He says, "If the host suits the fungus only in a limited degree, then no hypertrophy will result, and the latter will attain only to the formation of spermagonia. Let the host, however, be the one best suited to the fungus, then hypertrophy will result and aecidia be developed." He gives as evidence a series of experiments upon *Gymnosporangium*, in which the fungus developed to different degrees varying with the host used. FENSTLING (**22**), in discussing the effects of rust upon their hosts, comes to the same conclusion regarding the relation of the degree of change to the time of the fungus attack.

In the leaf-inhabiting fungi, so far as studied, the mode of attack seems to be through the aid of some substance injurious or stimulative to the host cells. Two lines of evidence are at hand. The protoplasm of cells outside of the tissue directly in contact with the fungus often becomes killed, and the cell walls become brown. It is difficult to see how such a condition could exist if there is no toxic substance produced. In the second place, most of the cells of the host tissue examined by the writer contained no trace of the fungus, yet, as already shown, the nuclei are often enlarged or become numerous, and the chloroplasts also are reduced in size. Here again, there must be a substance which is diffusible through the cell walls, which is stimulative to the nucleus or poisonous to the chloroplasts. No indications were to be found as to the origin of such a substance. It is possible that the host cells may have produced it as a defensive measure, and that in turn certain of the cells were killed by this toxic substance. On the other hand, it is possible that the fungus may produce such a

poisonous substance which directly affects the host cells, as WARD (**86**) claims for the *Botrytis* fungus that causes the lily disease. WORONIN (**93***b*) attributes in an inferential way the effect of *Sclerotina Vaccinii* upon the cowberry to the same process. TUBEUF (**80**) quotes from him thus: "Here a peculiar phenomenon is exhibited, the fungus exerts its injurious effects on the *surrounding tissues* of the host plant, then, having killed these, it utilizes them as food material." Thus the tissues are killed first, apparently even beyond the immediate vicinity of the fungus, and are later used for food. That the parasitic fungi upon leaves produce this toxic substance is more easily believed than that the host produces it, and is itself killed by the protective measure. The nature of the poisonous substance, whether a chemical organic poison or an enzyme, could not be determined in the material at hand.

Another interesting question, whose answer may be at least partly suggested, has to do with the strict limitation of certain leaf-inhabiting fungi, such as the shot-hole fungi. This strict limiting of the area of influence of the fungus is shown in the *Smilax* disease described earlier in the paper. At the margin of the diseased area the leaf cells are killed and turned a deep brown to black. This discoloration may be due to the excessive production of tannins, and if so, this would probably explain why the fungus proceeds no farther. BOKORNY (**5***b*) has found that tannins will inhibit the growth of fungi, and it seems quite likely that the production of tannins in the leaf finally stops the further growth of the fungus.

Summary

In the review of the previous work, it was found that many changes have been noticed in the organs and tissues of flowering plants. The cytologic changes, however, were especially emphasized. Very little work has previously been reported upon the effect of fungi on the cell contents of leaves, and the writer has shown that in such cells the nuclear and protoplasmic changes, which other workers have noted in cells of other plant organs attacked by parasites or under the influence of other destructive agents, also occur in leaf tissues when attacked by the parasitic fungi examined.

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